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COMPLIMENTARY

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ISOLATION AND CHARACTERIZATION OF A MANNANOLYTIC BACTERIUM

N.S. Mendoza, F. S. Cubol, E. G. Panerio and L. M. Joson[1]

ABSTRACT

A bacterium isolated from decomposing copra and identified as Bacillus subtilis was selected the most active mannan utilizing organism based on saccharifying activity and percentage degree of degradation of coconut residue. It also exhibited the highest enzyme activity when grown in mineral salts medium containing either coconut residue or locust bean mannan as sole carbon sources. The effect of pH and temperature ranges for activity of the crude enzyme were 5.0 to 6.0 and 50 to 55°C, respectively. Paper chromatography analysis suggests mannose as the major hydrolysis product of the crude enzyme.

INTRODUCTION

In coconut producing countries like the Philippines, large volume of coconut residues is discharged as by-product in both dry and wet processes of oil extraction from mature coconut meat. The carbohydrate composition of the coconut endosperm is 74% mannose, 21% dextrose and small amounts of arabinose and galactose (1,2). This indicates that the coconut meat is a rich source of mannans which are homopolysaccharides of D- mannopyranose residues.

The coconut meat or endosperm, however, has not been fully utilized for food and feed despite its high protein content (3) because of its high crude fiber content. It is therefore important to utilize the by-products from coconut meat either as residue or as copra meal not only for food or feed but also to develop further its utilization into more valuable products. One efficient method is to search for microorganisms which would hydrolyze the coconut residue polysaccharides to reduce its crude fiber content and thereby increase oil extractability. Moreover, it seems attractive to convert the residues to nutritive proteins by finding an organism capable of transforming the mannan into some metabolizable carbohydrates. This paper describes the identification and characterization of an active mannanolytic bacterium isolated from decomposing copra.

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[1] Industrial Technology Development Institute, Manila, Philippines

MATERIALS AND METHODS

Materials

Coconut meat residue was prepared from freshly grated matured coconut extracted of coconut milk using a homogenizer. The residue was washed with acetone followed by an ether-alcohol (3:1) washing and then dried overnight at 80°C. Mannan from coconut residue was prepared according to the procedure of Mukherjee and Rao (4). Other substrates and chemicals were of reagent grade available commercially.

Media

The isolation medium contained the same mineral salts as used by Pablova and Tin'yanova (5) and Jones and Ballou (6). Coconut meat residue (0.5%) was added as sole carbon source. Platings were done on the same medium except that coconut residue mannan was substituted for coconut residue.

Method of Isolation of Mannanolytic Bacteria

Decomposing copra samples and adjacent soil mixture (2.5 g) obtained from a coconut field in Nagcarlan, Laguna, Philippines were macerated and dispensed into 50 ml isolation medium and incubated with shaking at room temperature for 24 hours. One ml of the suspension was transferred to new medium and incubated under the same conditions as above. This process was repeated five times to enrich for coconut residue-utilizing bacteria, Dilutions of the supernatant were plated on mannan agar incubated at 37°C and colonies showing areas of clearing were observed.

Characterization and identification of the Organism

The mannanolytic isolate was characterized by standard biochemical and morphological techniques and Bergey's Manual was used for the identification.

Crude Enzyme Preparation

Cell-free supernatants containing the enzyme was obtained after centrifugation of the 24-hr culture broth of the organism grown in liquid medium containing the mineral salts mixture with 1% locust bean mannan.

Enzyme Assay

Locust bean galactomannan was used as substrate. Reaction mixture consisted of 0.5% substrate in 0.1M acetate buffer, pH 5.5 and enzyme in a final volume of 1.0 ml. Incubation was carried out at 37°C for 10 min. Reducing sugars as mannose were measured by Nelson-Somogyi method (7). A unit of enzyme activity is defined as the amount of enzyme that releases 1 umole of mannose per minute.

Gravimetric Assay of Enzyme Activity

The organisms were inoculated in flasks containing the mineral salts medium except that peptone was omitted and yeast extract concentration was decreased from 0.5 to 0.1%. Limited amount of coconut residue (0.1%) was added as sole carbon source and incubated at 37°C for 1 to 2 weeks in a reciprocal shaker. The degree of solubilization of the coconut residue was measured gravimetrically using the procedure of Lembeck and Colmer (8).

Paper Chromatography

Hydrolytic products from the reaction of the crude enzyme and locust bean mannan were analyzed by ascending paper chromatography after passing through anion and cation exchange resins to minimize streaking. The effluent was evaporated to dryness, resuspended in 1.0 ml water and approximately 20 ul were spotted on filter paper (Toyo Filter Paper No. 50). The chromatogram was developed for 24 hrs. with butanol-pyridine-water (6:4:3, by volume). Reducing sugars were detected after after dipping in silver nitrate-acetone solution.

RESULTS AND DISCUSSION

After a series of repeated enrichments and platings, seven out of 46 bacterial colonies that developed in the preliminary screening showed relatively high mannanase activity based on size of clearing zones formed on mannan agar. Attempt was made to select the best strain capable of utilizing mannan and results are shown in Table 1. Coconut meat residue was easily digested by strains No. 39 and No. 2. It seems that the physical treatment (ball milling) subjected to the coconut residue could be enough in altering the structures of the coconut polysaccharide fibers for it to be susceptible to enzymatic degradation. This suggests the potentiality of the substrate considering further that it is a waste product for SCP production.

Strain No. 39 also excreted the highest mannanase activity in the medium when either coconut residue or locust bean mannan was used as carbon source (Table 2). Thus, it is apparent that the enzyme is inducible as both carbon sources consist largely of (1-4)B-linked mannose residues. Although the organism grew well in the liquid medium without the carbon sources, insignificant amount of mannanase was produced.

The selected strain of mannan-utilizing organism is a small, motile, aerobic, spore-forming, gram-positive, rod-shaped bacterium. Its morphological and biochemical characteristics shown in Table 3 are identical with that of Bacillus subtilis described in Bergey's Manual.

Figure 1 shows the effect of pH and temperature on mannanase activity of the isolated strain. The pH and temperature ranges of 5 to 6 and 50 to 55°C, respectively were necessary for optimum activity.

Our chromatography of the hydrolysis products after 24-hr. incubation of locust bean mannan with the crude enzyme showed that the basic product is mannose. In addition to mannose, faint spot overlapping that of mannose was detected. This compound was not identified due to inavailability of higher mannooligosaccharides as markers. The results however indicate that the crude enzyme had substantial mannanase activity and that the hydrolysis attack results in production of mannooligosaccharides which are further degraded to mannose thereby being metabolized by the cell.

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Table 1. Degradation of coconut residue by mannanolytic bacteria

Strain No.	Initial Wt. mg	Degree of Digestion* Final Wt. mg	Solubilization %
18	51.5	26.6	48.4
9	50.7	17.5	65.5
20	51.4	24.3	52.7
11	49.8	25.8	48.2
14	49.2	24.2	50.8
39	52.0	13.1	74.8
2	51.2	17.5	65.8

*Degree of substrate digestion was measured gravimetrically after the isolates were inoculated into 50 ml mineral salts media containing 50 mg coconut residue as sole carbon source, and incubated at 37°C for 5 to 7 days.

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Table 2. Mannanase activities of the mannanolytic bacteria

Strain No.	Enzyme Activity*	
	Coconut Residue units/ml	Locust Bean Mannan units/ml
18	0.13	0.39
9	0.25	0.69
20	0.18	0.23
11	0.18	0.21
14	0.19	0.28
39	2.18	1.77
2	1.7	0.98

*The crude enzyme obtained from the 24-hr growth of the isolates in the liquid medium containing 1% of the carbon sources each (coconut residue and locust bean mannan) was assayed for mannanase activity as described in the Materials and Methods.

Table 3. Morphological and Biochemical Properties of Bacillus subtilis and Mannanolytic Bacterium

Property Morphological characteristics	B. subtilis	Isolate
Shape	irregular rod	irregular rod
Size (um)	0.6-0.8x1.3	0.8 - 1.3
Spores	+	+
Swollen sporangium	–	–
Motility	+	+
Gram stain	+	+
Biochemical characteristics		
Glucose	Acid	Acid
Lactose	Acid	Acid
Maltose	Acid	Acid
Sucrose	–	Acid
Arabinose	Acid	Acid
Xylose	Acid	Acid
Mannitol	Acid	Acid
Starch hydrolysis	+	+
Casein hydrolysis	+	+
Nitrate reduction	+	+
Indole production	+	+
Oxygen requirement	aerobic	aerobic
Catalase test	+	+
Utilization of citrate	+	+
Growth at pH 5.6	+	+
Voges Proskauer test	+	+
Methyl red test	+	+

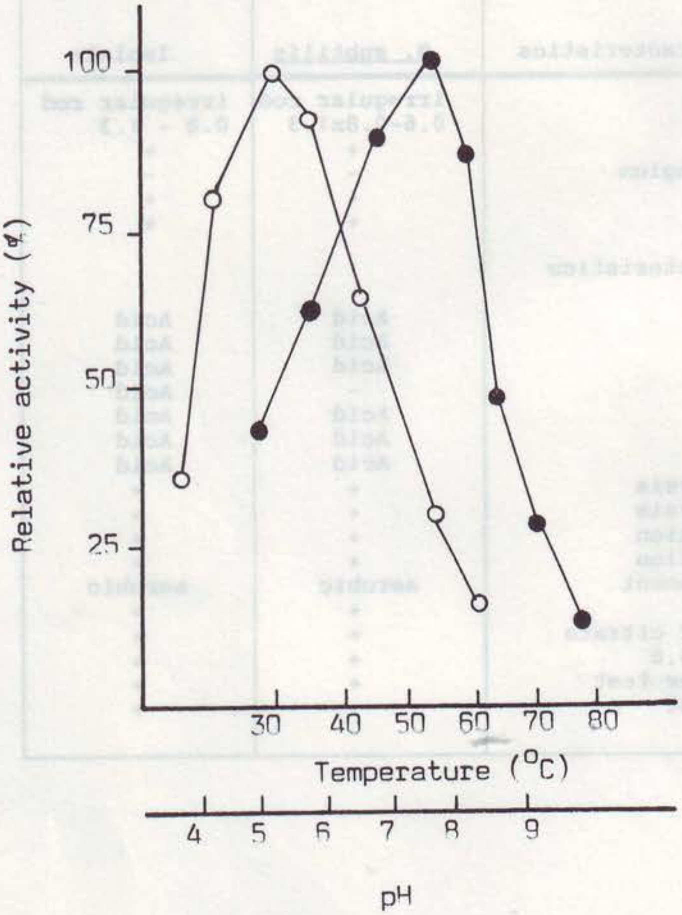


Fig. 1. The effect of temperature (●) and pH (○) on the mannanolytic activity of strain No. 39

UTILIZATION OF COCONUT HUSK: A COMPARATIVE INVESTIGATION OF THE ION EXCHANGE PROPERTIES OF COCONUT HUSK FIBER

PREM NATH MAINI[2] and JOSE H. SANTOS
Institute of Chemistry College of Arts and Sciences
University of the Philippines, Los Baños College, Laguna

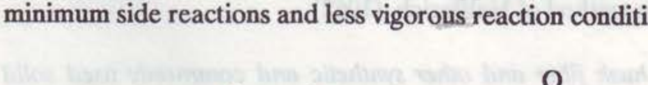
ABSTRACT

Mercury (II)-exchanged coconut husk fiber was employed for hydrolysis of 2-methyl-3-butyn-2-ol to 3-hydroxy-3- methyl-2-butanone in 71.58 and 51.95% yield by CH-100 and CH-40 mesh sizes of coconut husk fiber respectively, comparable to 74.09% yield by Dowex-50 resin. The cation exchange capacities of CH-100 and CH-40 mesh size of husk fiber were found to be 0.98 and 0.75 meq/g, respectively.

INTRODUCTION

The cation exchange properties of naturally existing cellulosic materials have been studied by various researchers (Masters, 1922; Ludtke, 1936; McLean and Wooten, 1939; Haymann and Ravinov, 1941) and they have shown that incorporated cellulosic materials exhibit cation exchange properties.

Ion exchangers are used as catalytic supports in chemical reactions. Several organic reactions are catalyzed by acids, alkalies or metal ions. They are employed directly or indirectly by exchanging them on the synthetically available ion exchangers. Newman (1953) studied the hydrolysis of terminal triple bond to ketonic form in the absence of, directly using and by supporting mercury (II) ions on Dowex-50 resin showing the advantage if indirectly using the support in terms of high major product yield, minimum side reactions and less vigorous reaction conditions.



$$-C \equiv C{-}H \xrightarrow{Hg^{+2},\ Hg^{+2}\text{-Dowex-50}} -\overset{\overset{O}{||}}{C}-CH_3$$

The advantage of indirectly using solid have been demonstrated by various examples of conversions of terminal triple bond to ketonic group (Gelbard, 1977; Yamawaki and Ando, 1979; Gree et. al., 1980; Lansbury et. al., 1980; Schick, 1982; Maini, 1986; Maini et. al., 1988). The role of Hg^{+2} and H+ ion has been documented (March, 1985). To date, no study was carried out to determine the efficiency and capacity of coconut husk fiber, although coconut fibers has been utilized for purification of water (Fraenkel, 1974) and in removal of heavy metal from waste water (Latif, 1989), which contains up to 43.44% of cellulose to act as a cation exchanger thus prompting this investigation.

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[2] Present Adress: P.N. Maini Consulting Chemist, PhilRice Pili Drive, UPLB Campus, College, Los Baños, Laguna.

MATERIALS AND METHODS

I. List of Reagents: 2-methyl-3-butyn-2-ol, Mercuric (II) oxide, Dowex-50 resin, Silica gel, Alumina, Florisil, Cellulose, Celite, and Molecular Sieves.

II. The investigation was carried out in four steps:

- Preparation of husk fiber.
- Determination of the cation exchange capacity of the husk fiber mesh.
- Preparation of mercurated husk fiber and other synthetic and commonly used solid supports.
- Hydration of 2-methyl-3-butyn-2-ol by the direct and indirect use of Hg^{+2} ion.

Preparation of husk fiber:

Mature brown husks were used to obtain the husk fiber. The fiber was separated from the coir dust by mechanical means and washed several times with water. The separated fibers were sun-dried for 24 hours and then cut into shorter pieces. These were further ground into 40 and 100 mesh sizes and subsequently dried in an oven at 95-100°C for 20 hours. The oven-dried fibers were stored in tightly closed botles.

Determination of the cation exchange capacity of the husk fiber mesh:

The ion exchange capacities of the prepared CH-40 and CH-100 fibers were determined by the volumetric method of Helfferich (1962).

Preparation of mercurated husk fiber and other synthetic and commonly used solid supports:

About 10 g each of the solid supports CH-40, CH-100, Dowex-50 resin, silica gel, alumina, florisil, cellulose, celite, and molecular sieves were treated with acid and mercury (II) solutions according to the procedure of Newman (1953).

Hydration of 2-methyl-3-butyn-2-ol by the direct and indirect use of Hg^{+2} ion.

The 5 g of 2-methyl-3-butyn-2-ol in 30 ml of methanol - water mixture (2:1) containing 3-4 drops of concentrated sulfuric acid was stirred with 5 g of mercurated husk fibers at room temperature for 24 hours. The same method was used for the other solid supports. The final mixture was filtered free from husk fibers (or other solid suports). The filtrate was diluted with 20 ml of water extracted thrice with 20 ml aliquots of chloroform. The combined chloroform extracts was washed thrice with 20 ml of water, and then dehydrated with anhydrous sodium carbonate. The chloroform was distilled

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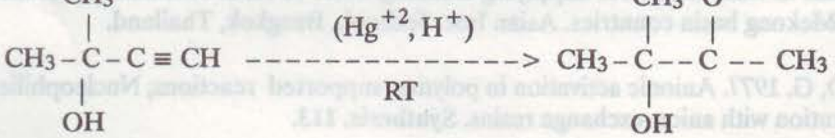
off and products from various reactions were collected at 138-142°C. The yield of the product obtained area on the basis of the reactant used. The reaction was carried out with mercury (II) alone, without mercury, and with mercurated solid supports. The product 3-hydroxy-3-methyl-2-butanone was identified by its infrared and proton magnetic spectra and 2,4-dinitrophenyl-hydrazone derivatives.

RESULTS AND DISCUSSION

The cation exchange capacity of two fiber mesh sizes CH-40 and CH-100 was determined to be 0.75 and 0.98 meq/g. The difference in the ion exchange capacities can be explained by their different mesh sizes. In the preparation of mercury (II) exchanged meshes, no physical or visual changes were observed (see Table 1).

The hydration reactions were carried out at ambient conditions to provide identical reaction conditions, since somesynthetic supports are unstable at high temperatures. The yields of the product 3-hydroxy-3-methyl-2-butanone, with various reaction reagents are shown in Table 2. The husk fiber untreated or treated alone with acid or mercury (II) alone failed to effect the reaction given below, which evidently shows the role of solid support in the reaction.



$$CH_3-\overset{\displaystyle CH_3}{\underset{\displaystyle OH}{C}}-C\equiv CH \xrightarrow[RT]{(Hg^{+2},H^{+})} CH_3-\overset{\displaystyle CH_3}{\underset{\displaystyle OH}{C}}--\overset{\displaystyle O}{C}--CH_3$$

Using Hg (II) ion at 1M concentration only led to a poor yield of 3.8%. Dowex-50 resin showed the highest yield, 74.09% owing to its known high cation exchange capacity of 4.9 - 5.2 meq/g. Curiously, CH-100-Hg^{+2} mercurated husk fiber despite its much lower exchange capacity gave a comparable yield of 71.28% showing that the husk fiber of CH- 100 mesh size is effective and efficient as an ion exchange support for this reaction.

The product 3-hydroxy-3-methyl-2-butanone was easily identified due to IR signal at 1730 cm^{-1} indicating the presence of a carbonyl group (– C –) and by the disappearance of the PMR acetylenic peak at 2.46 and appearance of an additional methyl signal at 2.26 for CH-40 and CH-100 mercurated husk fiber (see spectra I&II). Also, the 2,4-dinitrophenyl hydrazone derivative of 3-hydroxy-3- methyl-2-butanone from various reactions showed melting points in the same range as reported in literature (140 - 141$C) (see Table 3).

SUMMARY AND RECOMMENDATIONS

This investigation confirmed that cellulosic materials can be used as cation-exchangers even in their natural form as exemplified by coconut husk fiber. It was also found that particle mesh size affects the ion exchange capacity of the husk fiber.

The successful conversion of the given acetylenic compounds by Hg(II) treated coconut husk fiber in comparable high yield, proved that coconut husk fiber can be utilized as a low capacity ion-exchanger and solid support for the catalytic hydration of triple bonds.

However, in order to prove the general use of coconut husk fiber as a catalytic support material, or as a low capacity ion-exchanger, a detailed study of more terminal triple bonded compounds and other classes of catalytic reactions is necessary.

ACKNOWLEDGMENT

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Table 1. Mercuration of solid supports.

SOLID SUPPORT	OBSERVATIONS	DRYING TIME (DAYS)
CH-100-Hg^{+2}	No visible change; fibers were initially floating but sank after 24 hours	3
CH-40-Hg^{+2}	No visible change; fibers were initially floating but sank after 24 hours	3
Dowex-50	From yellow-brown, the supoort turned yellow-orange	3
Silica gel	No visible change	2
Alumina	No visible change	2
Florisil	No visible change	3
Cellulose	From white, the support turned darker	3
Celite	From white to dirty white	3
Molecular sieve	Turned yellow in color	2

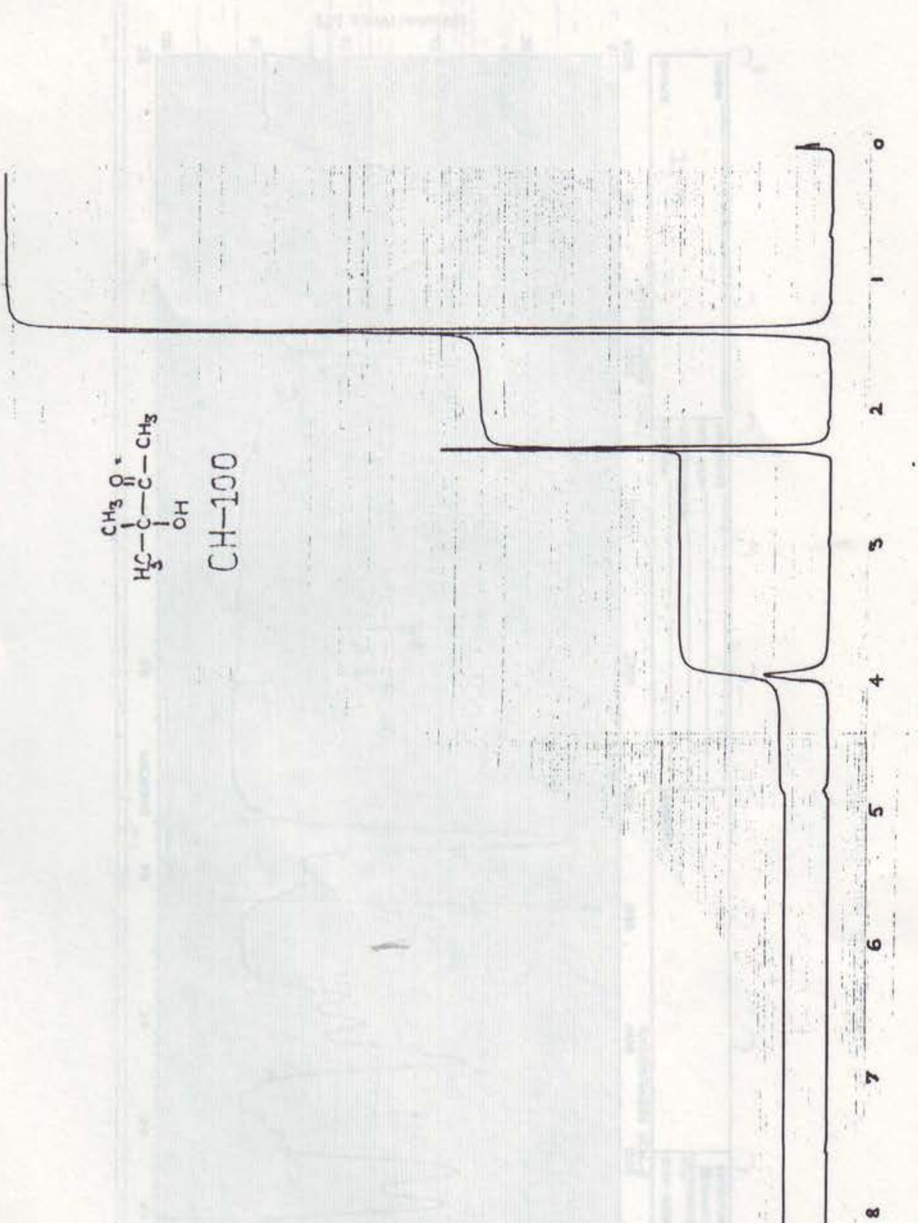
Table 2. Percentage yield of 3-hydroxy-3-methyl-2-butanone

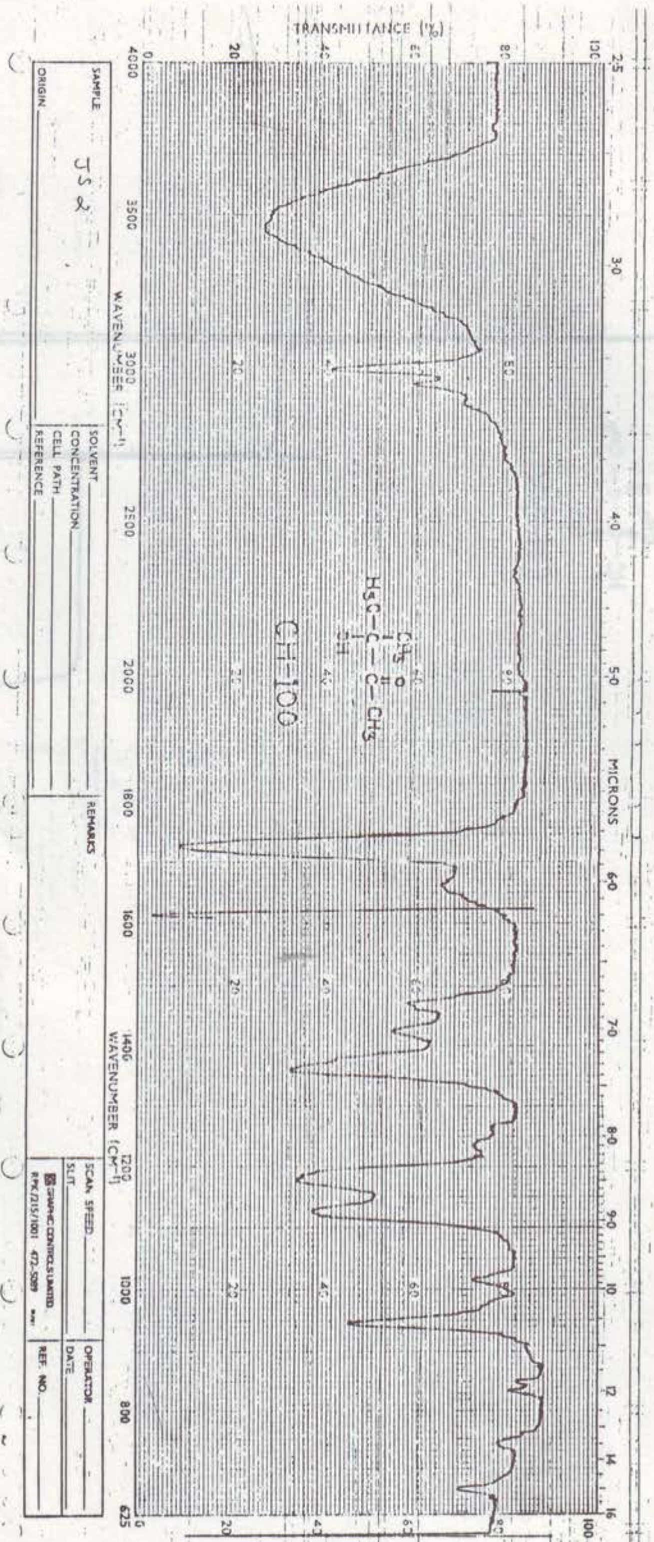
SOLID SUPPORT	PERCENT YIELD (MAX.)	CEC (MEQ/G)
CH-100-Hg^{+2}	71.28	—
CH-40-Hg^{+2}	37.15	—
CH-100-H^{+}	0.00	0.98
CH-40-H^{+}	0.00	0.75
CH-100	0.00	—
CH-40	0.00	—
Dowex-50	74.09	—
Silica gel	17.19	—
Alumina	3.86	—
Florisil	19.98	—
Cellulose	51.95	—
Celite	34.85	—
Molecular sieve	5.83	—
Pure HgO	3.87	—

*Cation exchange capacities (CEC) were determined, CEC for other materials cited in table are well known.

Table 3. Boiling points of 3-hydroxy-3-methyl-2-butanone (product) and melting points of resulting 2,4-dinitrophenylhydrazone derivatives with different solid supports.

SOLID SUPPORT	BOILING POINT OF PRODUCT, °C	MELTING POINT OF 2,4-DNP DERIVATIVE
CH-100-Hg^{+2}	140-142	138-139
CH-40-Hg^{+2}	140-142	137-138
Dowex-50	140-142	138-139
Silica gel	140-142	138-139
Alumina	138-140	137-139
Florisil	138-140	137-139
Cellulose	138-140	137-139
Celite	138-140	137-139
Molecular sieve	139-142	137-139
Pure HgO	140-141	138-139
Literature value	140-141	138-139


H3C—C(CH3)(OH)—C(=O)—CH3
CH-100
0
1
2
3
4
5
6
7
8
9
10


TRANSMITTANCE (%)
MICRONS
2.5
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10
12
14
16
0
20
40
60
80
100
WAVENUMBER (CM⁻¹)
4000
3500
3000
2500
2000
1800
1600
1400
1200
1000
800
625
CH3 O
H3C—C—C—CH3
OH
GH-100
SAMPLE JS 2
ORIGIN
SOLVENT
CONCENTRATION
CELL PATH
REFERENCE
REMARKS
SCAN SPEED
SLIT
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DATE
REF. NO.
GRAPHIC CONTROLS LIMITED

COLONIZATION AND ABUNDANCE OF ARTHROPODS ON CUCUMBER (CUCUMIS SATIVUS L.) AT VARIOUS STAGES OF PLANT GROWTH

EVELYN A. BERGONIA[3] and CLEOFAS R. CERVANCIA[4]

ABSTRACT

Insect pests, pollinators, and other arthropod associated with cucumber (UPL Cu-6) were monitored throughout their growing stages from March to May 1989 at the University of the Philippines , Los Baños, Laguna.

Colonization by insect pests, pollinators, parasites, predators and tourists associated with cucumber was generally influenced by plant age. In general, the arthropod population density increased from seedling to vegetative and reproductive plant stages and decreased as the crop senesced.

The colonization and temporal abundance patterns were discussed.

INTRODUCTION

The increasing demand for picking cucumber provides a good opportunity for local growers to expand cultivation. In spite of the crop's adaptability and fast growing market, cucumber production has not fully established because of inadequate supply of seeds (Gregorio, 1989). Low yields are usually attributed to insect pests, diseases, adverse climate and inappropriate cultural practices. Cervancia and Bergonia (1991) reported that inadequate pollination reduced fruit and seed set.

Many insects and other arthropods are associated with cucumber from seedling to maturity. Thrip infestations caused considerable yield reduction (Welter et al. 1990; Rosenheim et al. 1990). Aphid populations were monitored by Basky and Nasser (1989) where three species (Aphis goospil Glover, Aphis craccivora Kock and Myzus persicae Sulzer) were found vectors of virus causing diseases.

Screening for insect resistance has been done. Eight of 1160 cucumber lines showed resistance to picle worn Diaphania nitidalis Stoll (Wehner et al. 1985). Basky (1986) noted the importance of natural enemies in regulating aphid populations in Hungary and cautioned that chemical methods are dangerous to predators.

Monitoring the colonization and temporal abundance of arthropods will provide basal data for development of alternative management program for cucumber production. Such program should include regulation of pest population densities while conserving natural enemies, pollinators and tourists.

---

[3] Department of Entomology ,University of the Philippines, College, Laguna

[4] Institute of Biological Sciences, University of the Philippines, College, Laguna

MATERIALS AND METHODS

Land Preparation, layout and planting

A 600 m2 area in the Central Experiment Station, University of the Philippines at Los Banos was thoroughly harrowed and furrowed at a depth of 30 cmn. Seeds of a local picking cucumber variety, UPL Cu-6, were sown at a distance of 75 cm between rows and 40 cm between hills. Trellises made of wooden poles and straw were installed to support the growing plants. All the cultural practices recommended by Deanon and Angat (1984) were followed.

Observations were done from March to May 1989.

Colonization and abundance

Arthropods were monitored weekly during the growing stages of the crop. Samplings were mostly done early in the morning to avoid diurnal changes in species visibility. For floral visitors, observations were done during anthesis. Sampling was done by the following methods:

1. Sweep net sampling. The insect sweep net (38 cm diameter) was used to sample more mobile insects. A total of 10 sweeps were taken. Because of the presence of wooden trellises within the plots, brush strokes were done in between rows. The net contents were etherized and brought to the laboratory for identification.

2. Traps. Sticky broad traps (12 x 32 cm) painted yellow and red were placed horizontally above crop canopy. Four traps (2 yellow and 2 red) were placed equidistant in each plot. Ordinary grease was used as sticker. This was spread evenly on ethylene plastic cut just exactly the size of the board and fitted to the trap board. The plastic on the traps were changed every after three days. Kerosene was used as solvent to separate the insect from grease.

3. Per plant sampling. Five randomly chosen test plants, each from the 5 rows within each cucumber plot were examined for foliar pests. Individual counts were recorded from the first 5 fully opened leaves of each plant sample. The arthropods were grouped into three: 1) pests; 2) natural enemies and tourists; and 3) pollinators and flower visitors. Tourists are non-predatory species which have no permanent association with vegetation (Moran and Southwood, 1982).

RESULTS AND DISCUSSION

Pests

A total of 11 species colonized the crop (Fig. 1). The earlist colonists, thrips (Thrips palmi Kirkaldy) and squash beetles (Aulacophora similis Olivier) were observed at seven days after emergence (DAE). *A. similis* and *T. palmae* peaked at 14 and 42 DAE and 21 to 28 DAE, respectively.

At 14 DAE, two mite species (*Amblyseius sp.* and *Tetranychus sp.*), melon aphids (*Aphis gossypii Glover*) and leaf folder larvae (*Diaphania indica Saunders*) were added. All populations peaked at this stage and persisted until crop maturity.

Other species like 28-spotted beetle (Epilachna philippinensis Dieke), ladybird beetle (*Micraspis crocea Mulsant, mealybus (Ferrista sp.)* and *katydid (Phaneroptera furcifera Stal)* were not numerous and present only at certain stages of plant growth. They were not observed to cause economic damage to the crop.

Natural enemies and tourists

The species of parasites, predators and tourist which colonized the crop were listed in Table 1. Some important crop pests could have come from adjacent fields planted to corn, sugarcane, bitter gourd and legumes. The most numerous species collected belonged to Diptera (Culicidae, Dolichopodidae and Muscidae), followed by Coleoptera (Chrysomelidae) and Hymenoptera (Braconidae and Chalcididae). Other species observed were scarce.

Pollinators and flower visitors

Seven species were observed foraging on the flowers (Fig. 2). These were *Eumenes sp., Formicidae, Xylocopa sp., Catochrysops cnejus Fabricius, Chrosodeixis chalcites Esper, Diaphania indica Saunders and Parnara sp*. The populations were low with peaks at 21 and 28 DAE. Insect pests and mites, though represented only by 11 species were the most numerous (90%). This is because of the high population densities of mites, aphids and thrips. Their small size is compensated by high reproduction rates. It is also evident that these species have a wide habitat range because they were present from seedling stage to maturity. Thrips and aphids preferred younger plants as shown in their population peaks (21 and 28 DAE). Mite populations, however, peaked at both early (14 DAE) and late (42 DAE) stages. This shows that mites are less affected by plant age and structure.

The most diverse group was the natural enemy and tourists (42 species), although their population density was low (10%). No relationship, however, could be established between the populations of pests and natural enemies. It was noted, however, that the predatory coccinellid (Menochilus sexmaculatus) colonized the crop simultaneously with their host, A. gossypii. Predatory species are generalists that they prey on tourists as well. It was also difficult to separate the true predatory and parasitic species for identifications up to species level were not available. Most species were identified only up to families. The interactions, then, between prey and predator were not considered in this study.

There were only few species of flower visitors (1%). The populations were most abundant at the peak of flowering stages (21 and 28 DAE). Species visit the flowers for pollen, nectar or oviposition sites. Using Faegri and van der Pijl's (1979) criterion, only

Eumenes sp. and *Xylocopa sp.* were biotic pollinators. These species visited the blossom regularly and their visits constituted a regular part of their life activity. Moreover, they did not cause any damage to the flowers. Their foraging synchronized with anthesis. The absence of other bee species could be due to the destruction of their nesting sites especially in a developed agroecosystem.

Arthropod abundance significantly varied with season (Table 2).

Generally, arthropod populations were low at early stages of plant growth, increased with season, reaching peaks at mid vegetative and reproductive stages and decreased as crop senesced. It shows that plant stage and structure influence colonization and temporal abundance. At early plant growth stages, habitat is limited allowing only for herbivores and species seeking shelter and oviposition sites. As the plant grows, habitat also increases providing more food and shelter to the species. At reproductive stages, additional food sources are provided, like nectar and pollen grains.

The species present throughout the season were relatively few. This maybe due to the absence of natural vegetation near the site which can serve as potential source of colonists. Removal of uncultivated strips alters the succession of nectar and pollen yielding flowers foraged by pollinators and other species. Pesticide usage in the experimental area could have affected populations of arthropods specially the top predators.

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Table 1. Number of predators, parasites and tourists at different plant growth stages.

ARTHROPOD SPECIES	DAYS AFTER SEEDLING EMERGENCE 7	14	21	28	35	42
Aranae						
Lycosa sp.	0	0	0	0	0	42
Oxyopes sp.	0	0	0	0	0	33
Unidentified sp.	0	0	0	3	1	0
Coleoptera						
Chrysomelidae	3	14	8	33	4	3
Cassida circumdata	1	3	2	1	0	0
Monolepta bifasciata	5	0	3	2	1	0
Coccinellidae						
Menochilus sexmaculatus	0	16	4	0	4	2
Dermestidae	3	0	0	0	0	2
Tenebrionidae	1	1	11	0	2	7
Diptera						
Agromyzidae	18	15	32	17	11	19
Cecidomyidae	18	26	14	16	11	13
Chironomidae	2	8	4	0	0	0
Culicidae	88	74	45	38	54	72
Dolichopodidae	31	51	66	34	15	15
Drosophilidae	1	22	11	13	21	1
Ephydridae	7	12	13	14	19	11
Muscidae	32	29	18	17	28	11
Musca sp.	14	8	38	14	29	9
Phoridae	0	21	0	0	0	0
Otittidae	0	0	0	0	0	0
Psychodidae	5	1	0	0	0	1
Tipulidae	5	26	2	0	0	0
Hemiptera						
Dysdercus cingulatus	0	0	0	1	0	4
Miridae	2	1	2	0	0	0
Nezara viridula	0	1	0	0	0	0
Coreidae	0	0	1	0	0	0

Table 1. Continued

ARTHROPOD SPECIES	DAYS AFTER SEEDLING EMERGENCE 7	14	21	28	35	42
Homoptera						
Cicadellidae	9	4	5	0	1	0
Nephotettix sp.	1	0	1	0	0	0
Delphacidae	2	0	3	0	0	0
Fulgoridae	0	0	0	0	0	0
Ricania speculum	0	0	0	0	1	1
Hymenoptera						
Braconidae	3	4	2	0	0	2
Apanteles sp.	0	2	5	2	2	13
Chalcididae	6	1	11	7	2	8
Encyrtidae	0	0	0	1	0	0
Ichneumonidae	2	0	3	8	5	1
Platygasteridae	0	2	3	0	0	1
Pteromalidae	2	1	0	2	0	0
Odonata						
Coenagrionidae	1	0	0	0	0	0
Libellulidae	0	0	0	1	0	1
Orthoptera						
Acrididae	0	0	0	0	2	4
Gryllidae	0	0	0	0	1	1
Tettigoniidae	0	0	0	0	0	1

Table 2. Mean number of different arthropods collected at different days after seedling emergence of cucumber (March-May, 1989)[a]

DAYS AFTER SEEDLING EMERGENCE (DAE)	ARTHROPOD SPECIES Insect Pests	Pollinators	Tourists	TOTAL[b]
7	15.15	4.5	64.0	83.75 e
14	501.0	7.25	86.5	594.75 c
21	1730.25	12.25	76.75	1819.25 a
28	1059.25	14.25	55.5	1129.0 b
35	428.5	8.0	53.5	490.0 c
42	217.5	1.5	70.75	289.75 d
C.V. (%)				20.15
	3951.75	47.75	407.0	17624.0

[a] Based on 4 replicates.

[b] Means followed by the same letter in a column are not significantly different at 5% level using DMRT; based on square root (x + 0.5) transformation analysis.

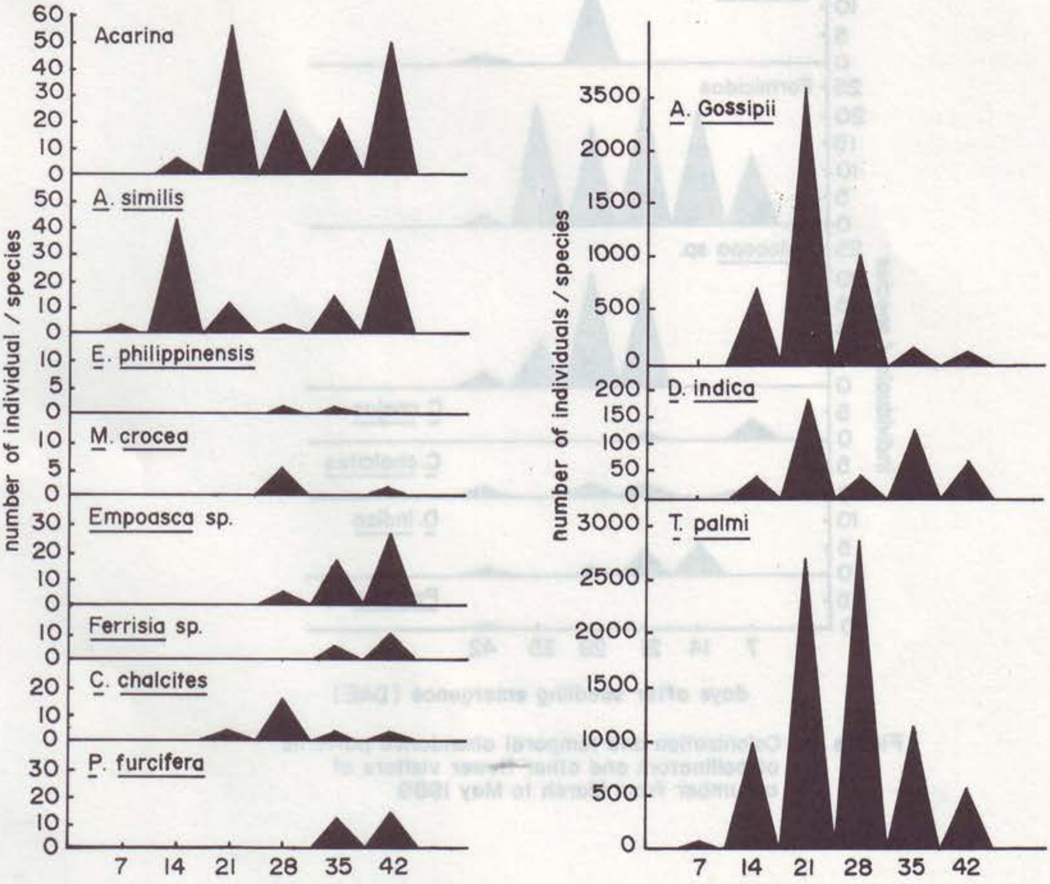

Figure 1. Colonization and temporal abundance patterns of mites and insect pests collected in cucumber (UPL Cu-6) from March to May 1989.

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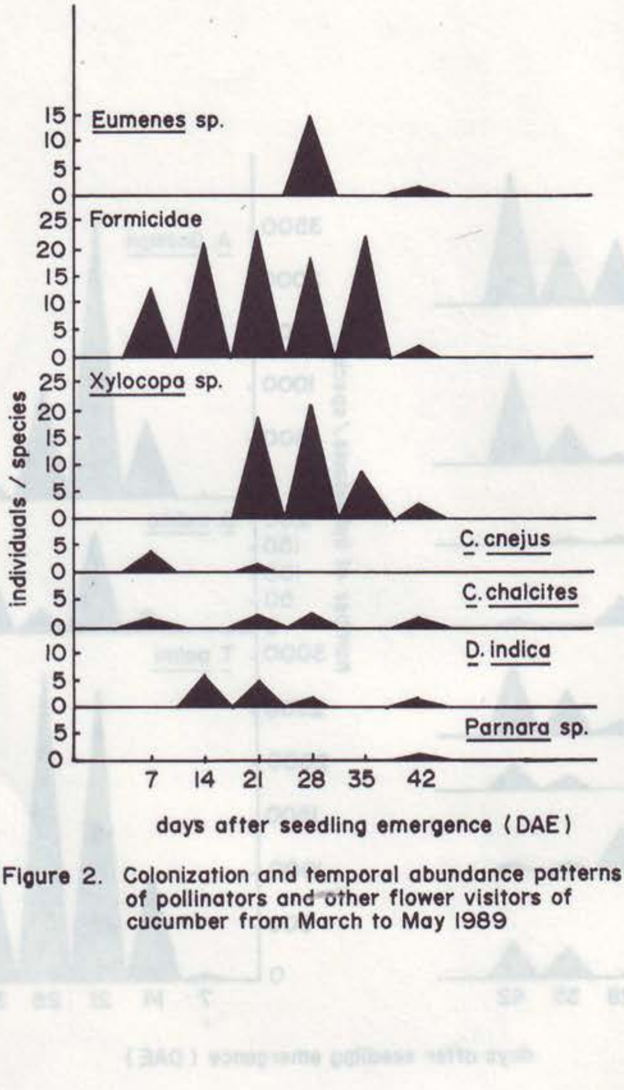

Figure 2. Colonization and temporal abundance patterns of pollinators and other flower visitors of cucumber from March to May 1989